

# **The Source, Cycling, and Behavior of Chromophoric Dissolved Organic Matter in Coastal Waters**

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Grant Number: N00014-05-1-0116

<http://www.marine.maine.edu/~wellsm/Homepage.htm>

## **LONG-TERM GOALS**

Our long term research goal is to ascertain the nature and magnitude of optical effects (absorbance / fluorescence / scattering) in surface seawaters associated with the production and cycling of marine colloidal organic matter. We are particularly interested in determining how these effects are driven or modulated by the productivity dynamics of phytoplankton and marine heterotrophic bacteria in coastal regions not directly influenced by high riverine inputs of terrestrially-derived materials.

## **OBJECTIVES**

Marine chromophoric dissolved organic matter (CDOM) imparts highly variable optical signatures in surface waters over short spatial and temporal scales for reasons not yet understood. While considerable research efforts currently are underway on the absorption and fluorescence characteristics of bulk CDOM and FDOM (< 0.2-0.7  $\mu\text{m}$ ), our primary objective is to follow the chromophoric signatures of different molecular weight fractions to ascertain how production (or allochthonous input) and removal of colloidal organic matter contributes to the high variability in bulk CDOM. A significant fraction (10-40%) of dissolved organic matter is colloidal in size (1-1000 nm), and this fraction is reactive to photodegradation and bio-degradation (to soluble substances) as well as aggregation (to large sinking particles). Marine colloids are inhomogeneous in their optical characteristics, and the abundance of colloidal matter can change rapidly in association with in-situ biological processes. Our objective is to determine to what extent the dynamic, opposing processes of production and degradation of colloidal CDOM influences the behavior of CDOM and FDOM in surface seawaters, and with that knowledge develop forecasting insights to the optical characteristics of nearshore waters.

<b>Report Documentation Page</b>			Form Approved OMB No. 0704-0188	
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1. REPORT DATE <b>2008</b>	2. REPORT TYPE	3. DATES COVERED <b>00-00-2008 to 00-00-2008</b>		
<b>4. TITLE AND SUBTITLE</b> <b>The Source, Cycling, and Behavior of Chromophoric Dissolved Organic Matter in Coastal Waters</b>			5a. CONTRACT NUMBER	
			5b. GRANT NUMBER	
			5c. PROGRAM ELEMENT NUMBER	
<b>6. AUTHOR(S)</b>			5d. PROJECT NUMBER	
			5e. TASK NUMBER	
			5f. WORK UNIT NUMBER	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> <b>University of Maine, School of Marine Sciences, Orono, ME, 04469-5741</b>			8. PERFORMING ORGANIZATION REPORT NUMBER	
<b>9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>			10. SPONSOR/MONITOR'S ACRONYM(S)	
			11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
<b>12. DISTRIBUTION/AVAILABILITY STATEMENT</b> <b>Approved for public release; distribution unlimited</b>				
<b>13. SUPPLEMENTARY NOTES</b>				
<b>14. ABSTRACT</b>				
<b>15. SUBJECT TERMS</b>				
<b>16. SECURITY CLASSIFICATION OF:</b> a. REPORT      b. ABSTRACT      c. THIS PAGE <b>unclassified</b> <b>unclassified</b> <b>unclassified</b>			<b>17. LIMITATION OF ABSTRACT</b> <b>Same as Report (SAR)</b>	<b>18. NUMBER OF PAGES</b> <b>13</b>
				<b>19a. NAME OF RESPONSIBLE PERSON</b>

## APPROACH

Our work over the last year have focused mainly on field studies aimed towards understanding how the optical characteristics of soluble and colloidal organic matter change under different phytoplankton growth conditions and vary among estuaries, coastal shelf regions and in offshore waters where phytoplankton blooms are stimulated. The sites we have used are the marine regions of the Damariscotta River estuary (Maine), the inner and outer continental shelf regions off the Juan de Fuca Strait and central British Columbia, and in the eastern subarctic Pacific. The latter two study regions were sampled during cruises of opportunity.

We employ two analytical approaches to study marine CDOM; a size fractionation technique and a statistical approach to categorizing the fluorescence characteristics of the bulk CDOM. We use Flow Field-Flow Fractionation (Flow FFF) to partition CDOM into a continuum of molecular weight sizes. Briefly, a flow field is applied at right angles to the channel flow within a shallow ( $\sim 200 \mu\text{m}$ ) ribbon-like chamber. Soluble fractions are driven through the membrane (1 KDa) on the accumulation wall, while colloidal components are driven to the accumulation wall. The resultant concentration gradient is opposed by diffusion (a function of colloidal size), resulting in colloids of different size being retained in different stream laminae. Unequal laminae velocities due to shear along the accumulation wall causes a well-defined separation of different colloidal size fractions according to the mean colloid proximity to the wall, which then is measured by UV absorption after the sample stream exits the flow chamber. Limitations of detection sensitivity has required that the entire colloid fraction be concentrated before analysis. We use on-line focusing, whereby seawater (1-50 ml) is “focused” at the head of the channel before proceeding with the normal analysis mode. A single wavelength detector (254 nm) has been primarily used for quantifying CDOM. Although this wavelength is lower than normally used for CDOM measurements, it provides the higher sensitivity needed to detect the colloidal sub-fractions in these highly diluted samples.

We measure the absorbance characteristics of colloidal CDOM by interfacing the channel outflow of FlowFFF to a 50 cm waveguide/CARY 50 spectrophotometer system to obtain multiple absorbance scans across the CDOM size continuum. The resultant slope data are contrasted to the absorbance spectrum of the bulk dissolved sample. The outflow from the waveguide is batch collected for analysis by excitation emission matrix spectroscopy (EEMS), with countour surface plots providing 3D fingerprints of fluorescent dissolved organic matter (FDOM) collected for different sample aliquots. EEMS effectively distinguishes among types of organic matter in seawater. In addition, absorbance scans are measured on each sample aliquot across the same excitation range to both characterize the absorbance characteristics and to enable the relative fluorescence efficiencies to be estimated. As a consequence, we are able to analyze the absorbance and fluorescence characteristics of different colloidal size fractions during a single sample run, and compare them with the characteristics of the bulk dissolved ( $<0.4 \mu\text{m}$ ) CDOM. The two objectives are to determine the extent that marine colloid cycling influences the optical signature of surface seawaters, and to compare EEMs signatures of different seawaters in relation to the colloidal contributions.

## WORK COMPLETED

This final report covers the partial year (4 months) of final support for the project. To provide a more complete perspective of the project achievements, we present an overview of the work completed during the last 16 months project.

Our primary field sampling site for studying the relationship between colloidal organic matter and CDOM in coastal waters was the marine segment of the Damariscotta River estuary, where we completed a long term sampling program to measure the seasonal variations in colloidal CDOM associated with measured changes in phytoplankton abundance. These measurements include size distribution of colloidal matter, the differences in spectral absorbance (~300-600 nm) and fluorescence signature (excitation emission matrix spectroscopy) with colloidal size. The purpose here was to establish a dataset on how the combined effects of non-point source terrestrial runoff and phytoplankton production influence the bulk and size distribution of CDOM and FDOM. We contrast this weakly estuarine, productive system with upwelling and non-upwelling shelf waters (Juan de Fuca eddy and associated waters off Vancouver Island and central British Columbia), and offshore waters of the iron-limited eastern subarctic Pacific using cruises of opportunity. Last year we participated in one major cruise to these latter regions. The purpose was to determine how colloidal CDOM characteristics and cycling change with phytoplankton blooms across diverse regions to ascertain whether general patterns exist. The coastal regions off Vancouver Island and central British Columbia provide an excellent contrast to the confined, phytoplankton and macroalgal influenced Damariscotta estuary because it contains discrete surface waters influenced by upwelling, high in-situ primary production, general continental and offshore inputs. The central British Columbia coast also is a region that creates mesoscale eddies which combine offshore and shelf waters before spinning off into the eastern subarctic Pacific. EEMS analyses were performed on-board the vessel on freshly collected samples. In addition to studying spatial variations, vertical profiles also were analyzed, providing an opportunity to examine the differences in the character of CDOM and FDOM upwelling with deep waters compared to that generated by phytoplankton production. Analysis of these data have not yet been fully completed though significant advances in understanding have evolved from the work done to date. These results provide valuable end-member characteristics for elucidating how much of the CDOM characteristics in nearshore coastal waters should be attributed to marine vs. terrestrial origins.

These field programs enabled us to continue expanding our database of EEMS from different coastal and oceanographic regions. We are statistically assessing the similarities and differences among colloidal size fraction and bulk CDOM using Principle Component Analysis. Variability within the data set is decomposed into a series of linear terms that can be used to evaluate the relative importance of changes in fluorescence bandwidth and wavelength shifting across size fractions and due to various processes such as dilution and bacterial or photochemical alteration. The intention here is to use these statistical groupings of optical characteristics according to water types (e.g., surface, mid-depth, deep, inshore, offshore) and locations (e.g., Gulf of Mexico, coastal Maine, Washington State waters, etc.) to develop a forecasting tool for predicting optical characteristics of a given nearshore region from knowledge of the physical mixing processes and the predominant source waters.

In this final year of the project we have developed a synthesis of the colloidal and bulk seawater experiments and field measurements. This synthesis provides an assessment of the production and loss of colloidal CDOM with respect to phytoplankton production and seasonal non-point source terrestrial runoff as well as the effects of biological and photochemical degradation on the optical characteristics of colloidal and bulk CDOM. During this time, we used Principal component analysis of colloid fluorescence to identify colloidal dynamics on a multi-year time scale in order to ascertain the relative and absolute changes in the contributions of these fractions and examine additional processes that may control their cycling. Principal component analysis was applied to EEMS results to examine statistical shifts in filtered unfractionated CDOM and colloidal CDOM ( $CDOM_c$ ) variability relative to such factors as photodegradation, phytoplankton bloom dynamics, precipitation events and tide. Statistical

analyses of colloidal fluorescence dynamics will continue, and we predict that understanding colloidal variability will be fundamental for achieving predictive insights to marine CDOM behavior.

Further efforts were focused on the operational capabilities of the newly acquired asymmetrical 4F system. Significant progress was made towards developing stable, repeatable acquisition of colloidal distribution data from filtered natural CDOM samples. In tandem with further examination on CDOM colloidal optical properties, work will continue towards coupling successful A4F colloid separations with the MALLS detection system. The aim of MALLS investigation of colloidal material is to investigate the theory that colloids contribute significantly to optical scattering processes in coastal and marine environments by establishing the significance of colloidal backscatter.

## RESULTS

We focus on three central hypotheses in this report.

Hypothesis 1 —The size distributions of colloidal CDOM vary seasonally in conjunction with pre- and post-bloom stages and these patterns contrast with times when terrestrial runoff is significant.

1. The smallest of the colloidal CDOM fractions (~1-18 kDa in size) is highly labile with very short residence times in the dark (hours), most often has a protein-like fluorescence character, but is maintained at elevated concentrations during high phytoplankton conditions suggesting it is maintained by a dynamic balance between production and degradation/loss.
2. The major source of this small colloidal fraction is marine phytoplankton during spring, summer and fall. In early winter, there is a sharp increase in small colloid CDOM abundance associated with runoff, but the residence time of this material is very short, indicating that the rapid removal of this matter was not replenished by in-situ processes (in contrast to phytoplankton produced matter). This runoff-associated increase in colloidal CDOM was not observed in early spring, indicating that seasonally dependent terrestrial processes must be taken into account when modeling the contributions of small colloidal CDOM to the optical characteristics of seawater.
3. The small colloidal CDOM associated with terrestrial inputs had similar optical characteristics (based on EEMS and spectral slope) to that associated with phytoplankton production. However, the bulk (unfiltered) CDOM characteristics changed significantly. In other words, the terrestrial effects on marine CDOM in this region are not reflected across the broad spectrum of CDOM sizes.
4. The small colloidal CDOM also is highly susceptible to photochemical degradation while larger colloids phases are comparatively resistant.
5. The large colloidal CDOM fraction (~18-350 kDa) is much less labile (i.e., is relatively stable in the dark) and it has a more humic-like optical signature (EEMS and spectral slope). The persistence of this larger colloidal CDOM in coastal waters then is indicative of a low rate of supply.
6. The abundance of the large colloid CDOM fraction also followed the seasonal distribution of chlorophyll concentrations, indicating that in these coastal waters in-situ processes also were the primary source of this CDOM; there was little increases in its abundance during periods of high runoff in early winter. Overall, this fraction is more stable chemically and it has less dramatic fluctuations in abundance and optical characteristics over the seasonal cycle.

**Interpretation:** A sub-fraction of CDOM having unique optical characteristics has the potential to change rapidly, particularly early in the season before the spring bloom, and in the fall after phytoplankton production begin to drop. Abundances during late spring (after the spring bloom) and summer are relatively closely tied to total chlorophyll concentrations. However, the abundance of colloidal CDOM can fluctuate wildly in winter, with increases being associated with increased runoff but rapid (days) return to low levels when runoff drops. The larger colloidal CDOM fraction was better correlated to chlorophyll concentrations indicating that predicting the contribution of this fraction to CDOM signatures in coastal surface waters might be achievable by remote sensing.

Hypothesis 2 — Photochemical degradation of CDOM in soluble and colloidal size fractions proceeds non-uniformly, with soluble and small colloidal chromophores being the most susceptible to photolysis.

1. This hypothesis has been verified in replicate experiments showing that the small colloidal CDOM fraction is more susceptible to photodegradation, as measured by changes in CDOM absorption and increases in S, than larger colloidal CDOM size fractions.
2. Photodegradation of colloidal CDOM is not spectrally uniform, as measured with EEMS. Loss of protein-like fluorescence fractions is more prevalent than loss of the humic portions of the spectra.
3. Paradoxically, while the larger colloidal CDOM fractions show photodegradation under full sunlight, the remaining colloidal CDOM has a *lower* spectral slope (i.e., opposite that seen in bulk CDOM and in the small colloidal CDOM fraction). These changes may reflect enhanced absorption of low molecular weight substances, or the photochemically-enhanced photochemical fragmentation of colloidal CDOM larger than quantified with our FIFFF method.

**Interpretation:** Photochemical processing of colloidal CDOM proceeds differently than truly soluble colloidal fractions. Predicting these impacts on the optical signature of surface seawaters should depend on the seasonally-dependent abundance of colloidal CDOM associated with in-situ phytoplankton blooms.

Hypothesis 3 — The fluorescent and absorption signatures of near surface coastal waters can be predicted based on knowledge of the source water masses and their optical characteristics.

1. Our sampling to date shows that CDOM fluorescence data (EEMS) provide a statistically significant fingerprint of different water masses within the same geographical region when using Principal Component Analysis on a large enough database to achieve statistical significance.
2. The temperature and salinity profiles in coastal upwelling waters off the Strait of Juan de Fuca and Washington State showed the presence of three distinct water masses in the near surface region (<50 m). PCA analyses identified unique optical characteristics within EEMS measured in these water masses that are consistent with the broader database for surface, mid-depth and deep ocean waters.
3. PCA analysis for Damariscotta River Estuary samples indicate that the CDOM and CDOM<sub>c</sub> in Damariscotta River estuary reflects a fluorescent signature similar to marine transitional waters (Coble, 1996) identified by previous analyses (Boehme et al., 2004). These findings demonstrate that CDOM chemistry along this extent of the Damariscotta River is significantly impacted by intrusion of marine waters rather than terrestrial, or benthic processes.

4. Application of PCA analyses to the seasonal distribution of small colloids (<18 kDa) for the years 2004-2006 a pattern of seasonal CDOM and CDOM<sub>c</sub> emerges. Clustered PC1 and PC2 values for the month of June 2005 indicate that CDOM during this bloom was optically distinct relative to that in August 2005. The distinction between June and August fluorescence properties could reflect the lack of fresh bloom-related CDOM input. By October, environmental conditions had significantly altered due to high regional rainfall resulting in statistically altered colloidal and CDOM fluorescence properties towards that of previously identified riverine CDOM.

**Interpretation:** If different water masses indeed have statistically identifiable optical features, and the bulk of these features are comparatively stable, then the optical characteristics of a given coastal water column can be predicted with knowledge of the physical oceanographic conditions (mixing of water masses) and the optical characteristics of the source waters. These, in turn, will be modulated in the photic zone by phytoplankton processes as described above.

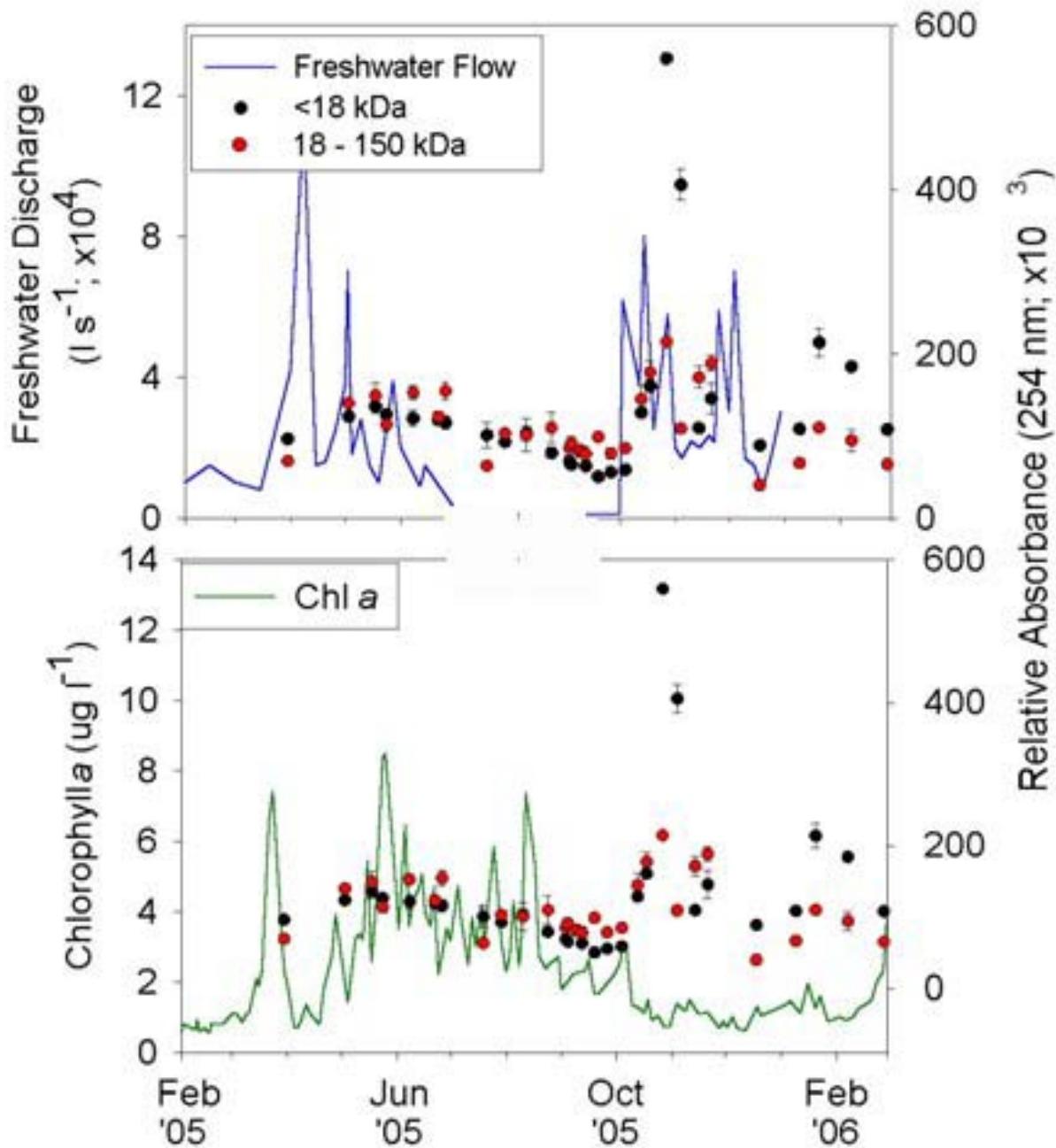
## IMPACT/APPLICATIONS

These findings address two unknowns surrounding marine CDOM; identifying the biogeochemical factors regulating the composition and spectral characteristics of CDOM, and the goal of predicting the optical characteristics of coastal waters over short (1 week) time intervals. We made significant progress in both of these areas. Better definition of the coupling between CDOM and colloidal processes and, in turn, the linkages between colloidal composition and the contributions of phytoplankton-associated production and small terrestrial inputs (relative to large river systems), provides key insight to the factors influencing CDOM abundance and character. Combining the use of excitation emission matrix spectroscopy with statistical database analysis tools (Principal Component Analysis) provides evidence that the optical characteristics of near surface waters may be predicted from knowledge of the source waters, their respective optical signals, and the general oceanography of a given coastal region. Though preliminary, these data to our knowledge provide some of the best insights on how to gain the predictive capability sought by scientists to assist in the remote characterization of spectral components of surface coastal waters.

The picture that emerges from our work is that a significant fraction of the CDOM and FDOM component in different seawaters are associated with the marine colloidal phase, and this fraction is a dynamic pool that can change over time scales ranging from days to months. These changes are associated with phytoplankton production and unidentified processes linked to freshwater influences in coastal waters. Colloidal CDOM optical characteristics, including both spectral absorption and fluorescence via excitation emission matrix spectroscopy (EEMs), vary as a function of size, likely due to rapid cycling by microbial processes. In addition to biological processes, exposure to sunlight leads to non-uniform degradation across the size spectrum of colloidal CDOM. Exposure-dependent effects reversed the expected trends of the spectral slope of the remaining CDOM. These results provide a rich backdrop of data from which to develop predictive insights on marine CDOM input, removal and cycling in nearshore waters removed from overwhelming inputs of terrestrially-derived CDOM.

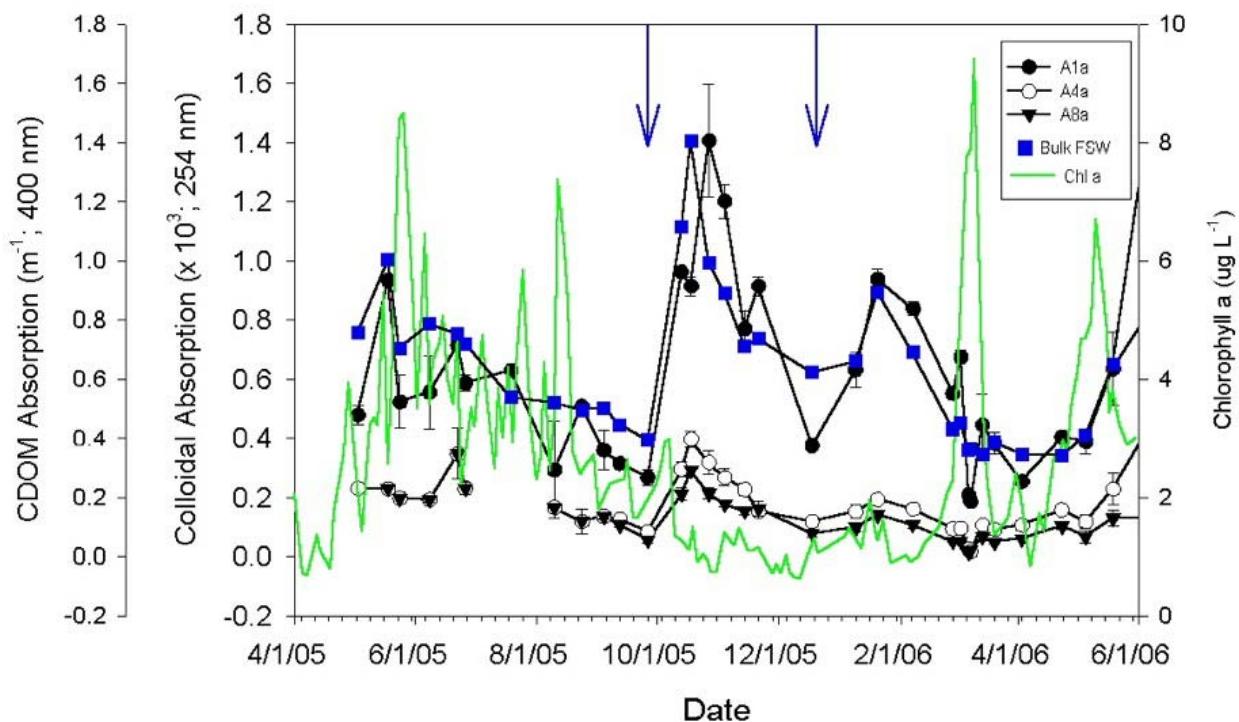
## RELATED PROJECTS

None



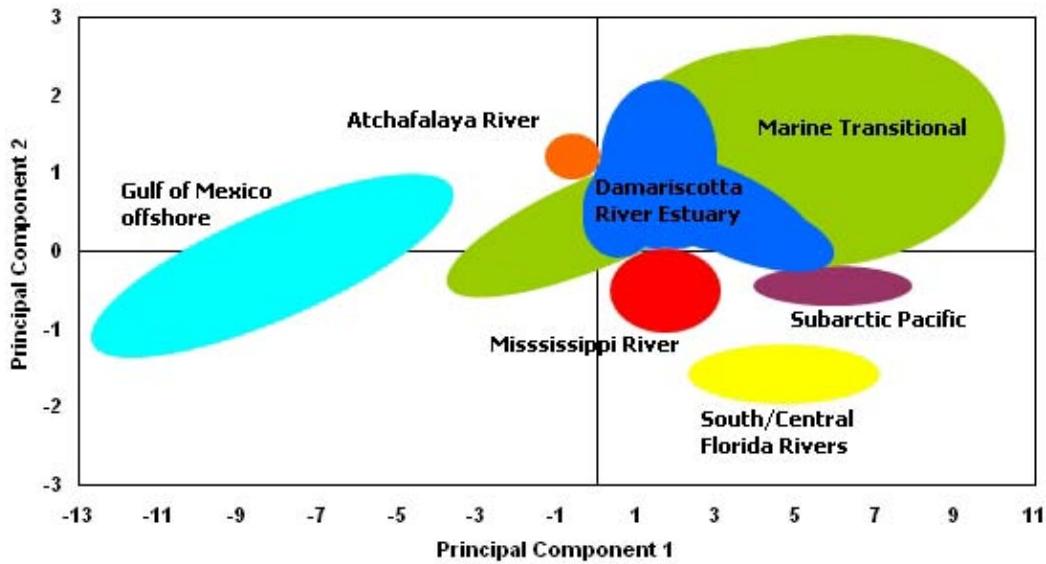
**Figure 1.** The relationships between small (<18 kDa) colloidal CDOM (black circles), large (18-150 kDa) colloidal CDOM (red circles) and freshwater discharge from the Sheepscott River, adjacent to the Damariscotta River estuary (Top Panel) and surface chlorophyll concentrations (Bottom Panel).

[The relative abundances of small and large colloidal CDOM are similar during the spring, summer and early fall, tracing the temporal pattern in chlorophyll concentrations (bottom panel), but not freshwater discharge (top panel). In late October, river discharge increased sharply, coincidence with a decrease in chlorophyll concentrations. Colloidal CDOM concentrations jumped significantly in conjunction with the fall runoff, and became much less stable compared to earlier in the season.]



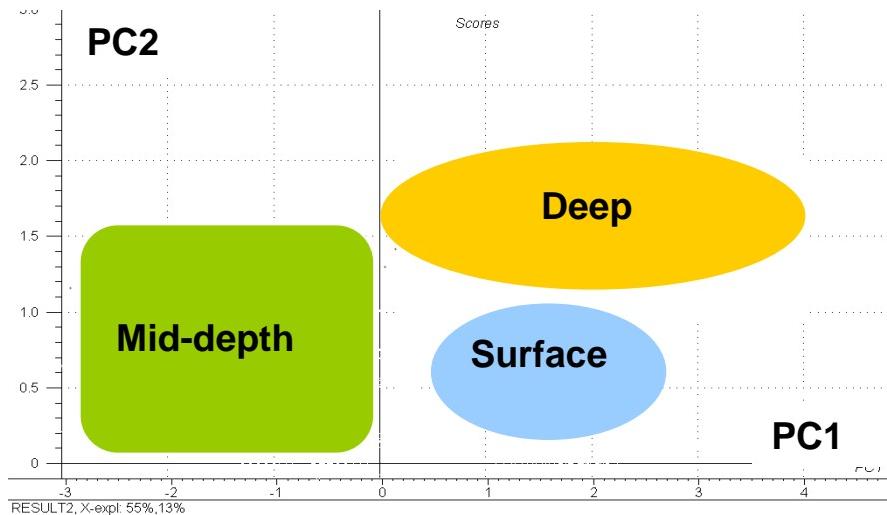
**Figure 2.** Changes in CDOM measured in bulk seawater (at 400 nm) and three colloid size fractions (A1 - < 5 kDa; A2 – 5-10 kDa; A3 – 10-15 kDa) in relationship with chlorophyll concentrations (green line) and total dissolved CDOM (blue squares). Marked high precipitation events are depicted by the vertical arrows.

[The annual cycle of bulk (unfiltered) and colloidal CDOM tend to follow that of chlorophyll concentrations in spring and summer, but increase sharply during winter months. The smallest colloidal phase (<5 kDa) followed closely the abundance of bulk CDOM, while larger colloidal CDOM had lower overall concentrations and displayed different temporal patterns in abundance.]



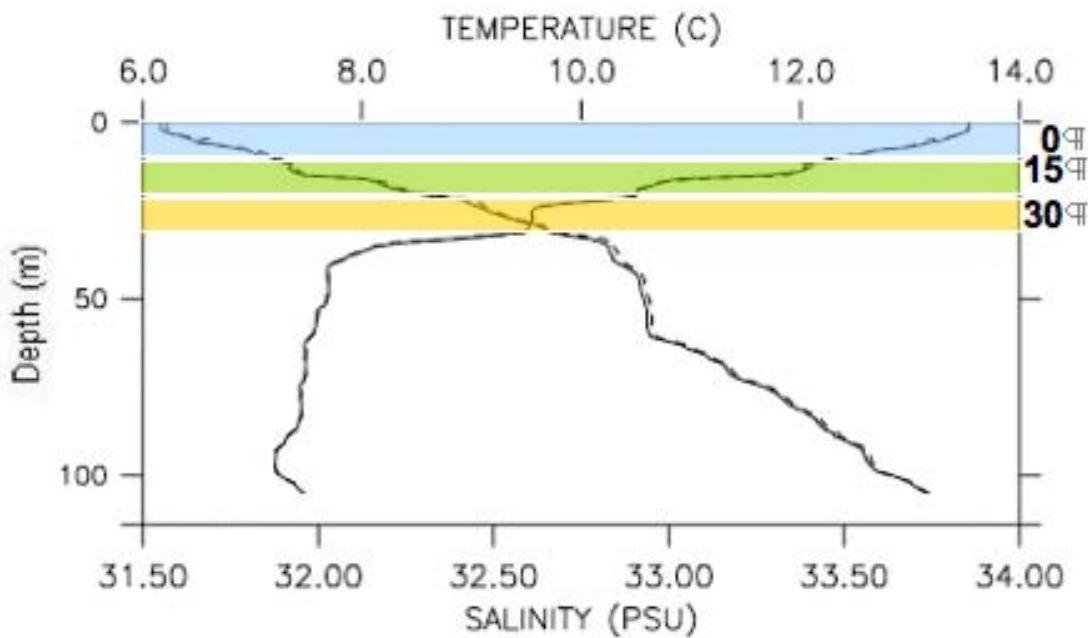
**Figure 3.** Principal Component Analysis plot showing the breakout distribution of EEMs data according to their statistical associations or differences.

[Statistical grouping of waters having similar fluorescence characteristics show marked degrees of unique signatures as indicated by non-overlap of statistical blots. In theory, these plots can be used similar to oceanographic T/S plots to determine relative mixing ratios of regional members based on their optical characteristics.]



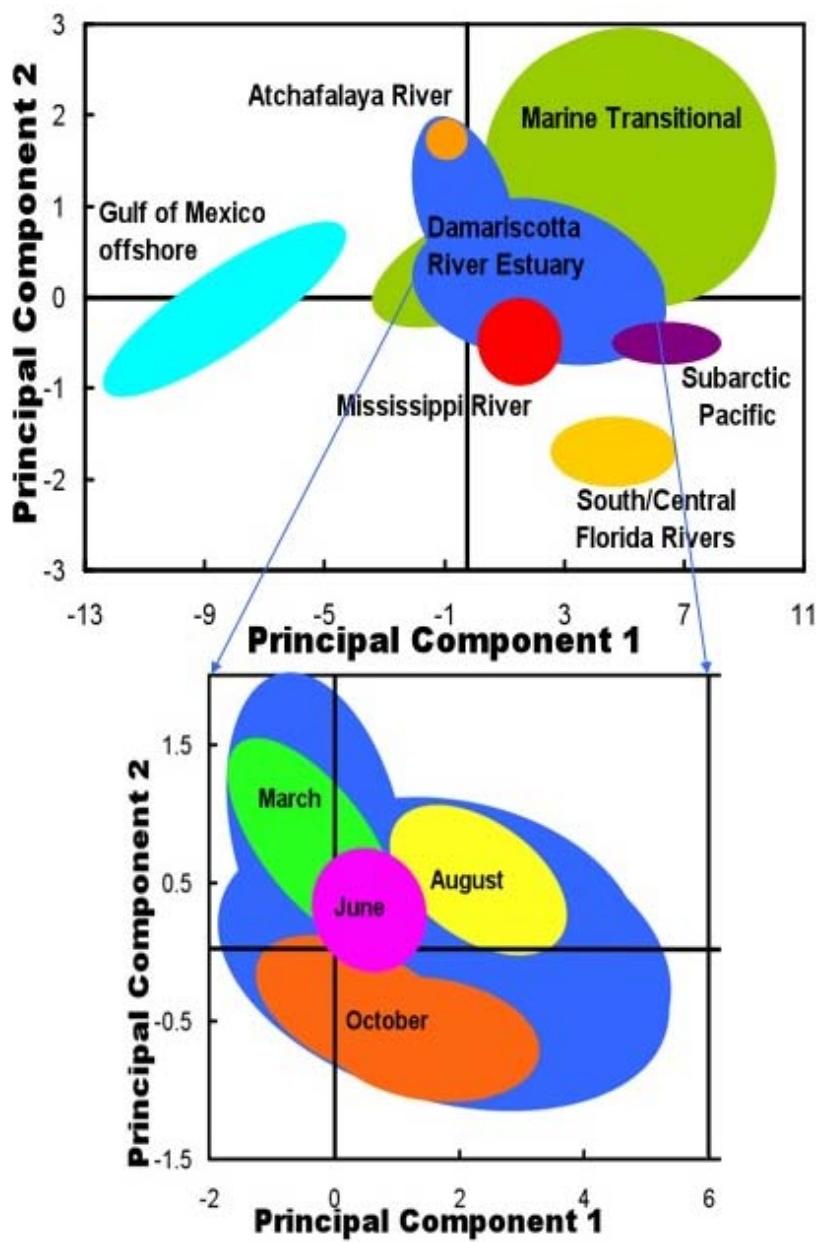
**Figure 4. Component Analysis score plot for a subset of samples collected from the Juan de Fuca eddy region off the Washington coast.**

[The regions identified above display the separation in statistical space for EEMs obtained from surface, mid-depth and deep ocean waters in this general coastal region. The clear separation suggests that it may be possible to predict the optical characteristics of coastal waters based on the relative mixtures of their source waters.]



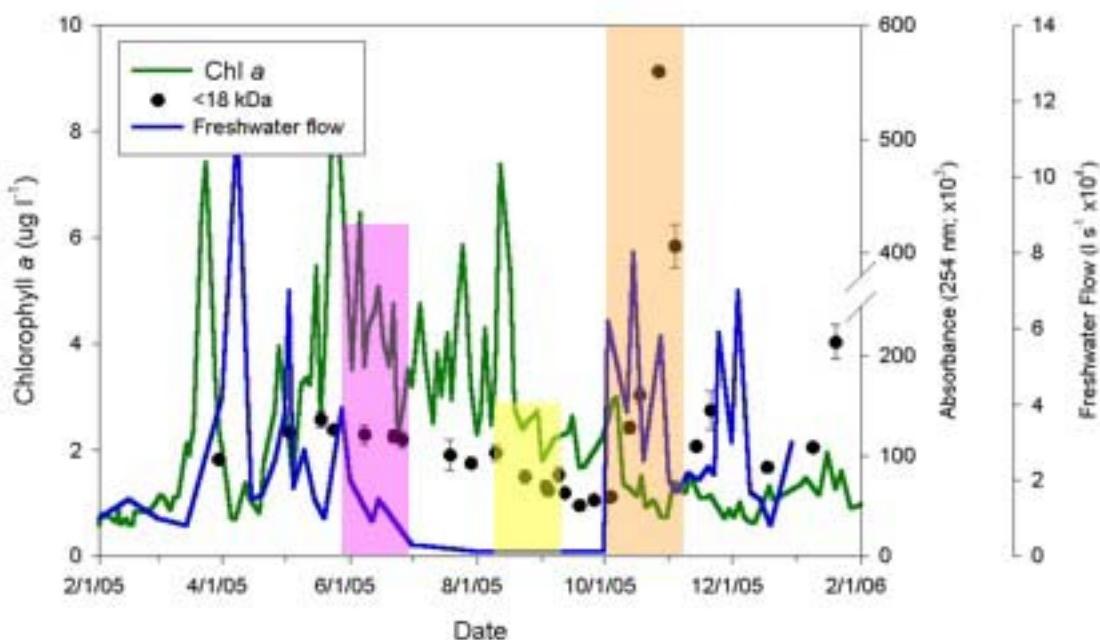
**Figure 5. Depth profiles of temperature and salinity in nearshore Juan de Fuca waters overlaid with the EEMs-derived indications of the source waters**

[Profiles of temperature and salinity show the presence of three broadly distinct water types in the upper 30 m of this upwelling region. PCA statistical groupings of surface, mid-depth and deep waters obtained from numerous samples in this region suggest that these water types can be correctly assigned by their optical characteristics. ]



**Figure 6.** Results of PCA analysis for Damariscotta River Estuary samples relative to regional CDOM distributions, reflecting statistical comparison of approximately 700 samples. The exploded view shows the shifting fluorescence of colloidal CDOM during key seasonal events, identified by the months in which they occur.

[The scores plotted for PC1 and PC2 indicate that the CDOM and CDOM<sub>c</sub> in Damariscotta River estuary reflects a fluorescent signature similar to marine transitional waters (Coble, 1996) identified by previous analyses (Boehme et al., 2004). This observation is consistent with the elevated salinities for this sampling site (>29 psu across all seasons). The finding demonstrates that the CDOM chemistry along this extent of the Damariscotta River is significantly impacted by intrusion of marine waters rather than terrestrial or benthic CDOM sources.]



**Figure 7.** Seasonal shifts in chlorophyll a concentrations, river flow and abundance of small marine colloids ( $<18\text{ kDa}$ ) in the Damariscotta Estuary with overlays identifying the time frames related to clustering of fluorescence data observed in score plots obtained by PCA, and relate to their respective clusters in Figure 6 by color.

[Results show that the PCA determined fluorescence characteristics with respect to the PCA components PC1 and PC2 change in conjunction with the time of the year, representing insight to the seasonal changes in the composition of the colloidal CDOM.]